

TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije  
Docket No.: 2183-6217US

1/13

| Constructs       |      |      |      |      |      |      |       | Deletion<br>(nucleotides) | pABV<br>number | M-<br>expression | N-<br>expression |
|------------------|------|------|------|------|------|------|-------|---------------------------|----------------|------------------|------------------|
| 5'UTR-<br>ORF1ab | ORF2 | ORF3 | ORF4 | ORF5 | ORF6 | ORF7 | 3'UTR | -                         | 437            | +                | +                |
| ///              |      |      |      |      |      |      | AAA   | Δ 11788-14139             | 594            | -                | +                |
| ///              |      |      |      |      |      |      | AAA   | Δ 14585-14984             | 521            | - <sup>D</sup>   | -                |
| ///              |      |      |      |      |      |      | AAA   | Δ 11788-14584             | 664            | -                | +                |
| ///              |      |      |      |      |      |      | AAA   | Δ 14985-15111             | 668            | - <sup>D</sup>   | -                |

<sup>b)</sup> Identical results were obtained in IPMA using MAbs against GP<sub>3</sub> and GP<sub>4</sub>

Fig. 1A

TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije

Docket No.: 2183-6217US

2/13

| Constructs | Deletion<br>(nucleotides) | PABV<br>number | M-<br>expression |
|------------|---------------------------|----------------|------------------|
|            | -                         | 437            | + <sup>1)</sup>  |
|            | Δ 14588-14936             | 605            | -                |
|            | Δ 14588-14885             | 604            | -                |
|            | Δ 14588-14786             | 603            | -                |
|            | Δ 14588-14687             | 602            | -                |
|            | Δ 14588-14642             | 624            | +                |
|            | Δ 14599-14642             | 625            | +                |
|            | Δ 14588-14600             | 626            | + <sup>1)</sup>  |
|            | Δ 14938-14980             | 638            | + <sup>1)</sup>  |
|            | Δ 14887-14980             | 637            | +                |
|            | Δ 14788-14980             | 636            | +                |
|            | Δ 14686-14980             | 635            | +                |
|            | Δ 14643-14686             | 631            | -                |
|            | Δ 14643-14676             | 632            | -                |
|            | Δ 14643-14664             | 633            | -                |
|            | Δ 14643-14652             | 634            | +                |
|            | Δ 14653-14686             | 696            | -                |
|            | rescue of 696             | 730            | + <sup>1)</sup>  |

<sup>1)</sup> Identical results were obtained in IPMA using MA b 122.17 against N

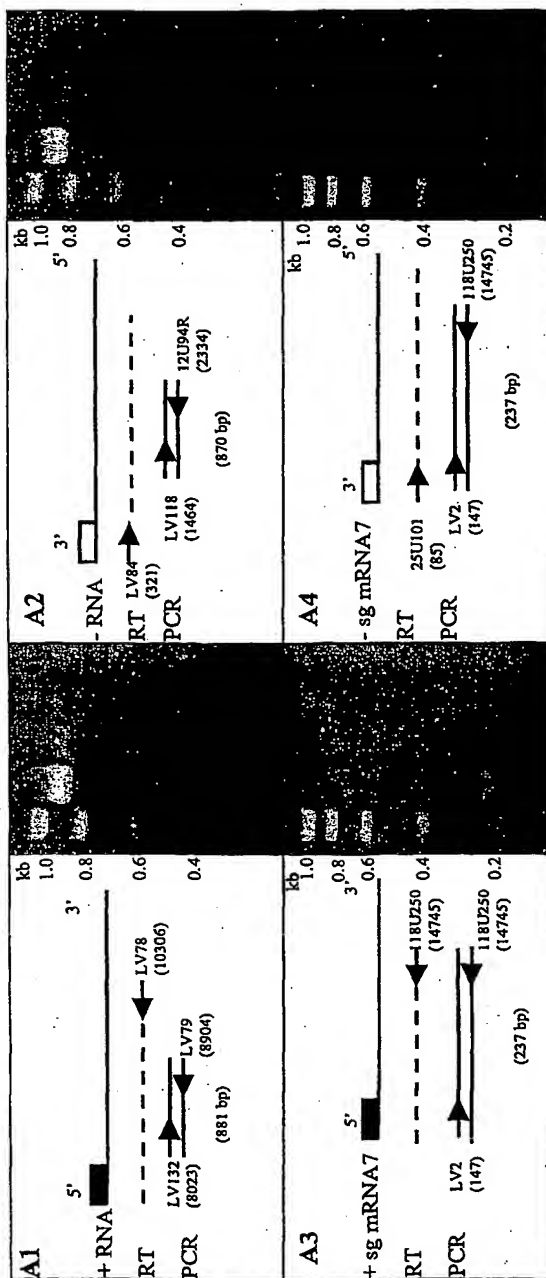
Fig. 1B

TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije  
Docket No.: 2183-6217US

3/13

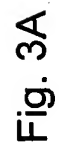
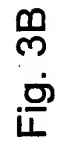
Fig. 2



BEST AVAILABLE COPY

Inventor: Monique Helene Verheije  
Docket No.: 2183-6217US

4/13



TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije  
Docket No.: 2183-6217US

5/13

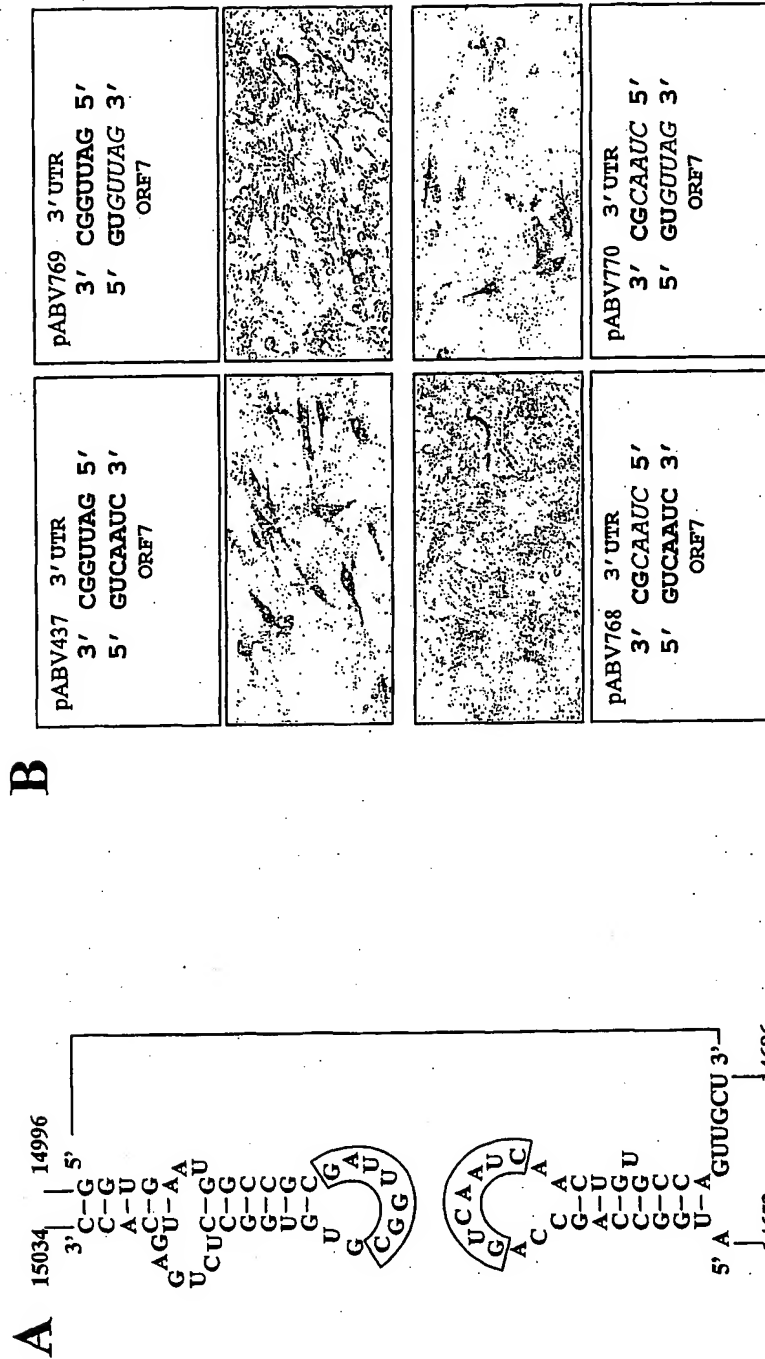


Fig. 4

BEST AVAILABLE COPY

TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije

Docket No.: 2183-6217US

6/13

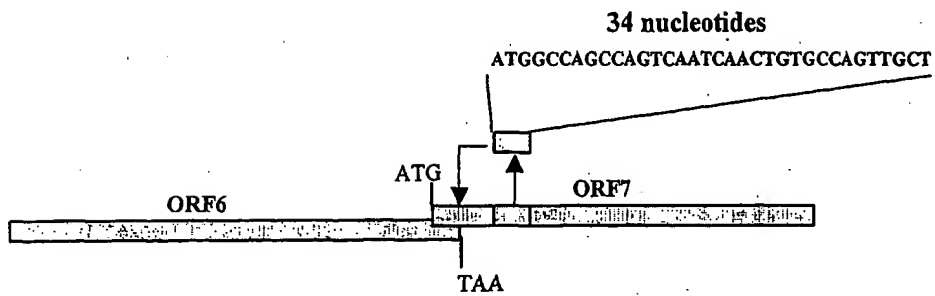


Fig. 5

7/13

A

```

LV      : MAGKNQSQKKKSTAPMGNGQPVNQLCQLLGAMIKSQRQ---QPRGGQAKKKKPEKHPFLAEDDDIRHH : 67
VR2332 : MPNNNGKQQKRRK-----KGDGQPVNQLCQMLGKIIAQQNQSRGKGPGKKNKKKPEKHPFLATEDDDVRHH : 66

```

```
LV      : LTQTERS L C L Q S I O T A F N Q G A G T A S L S S G K Y S F Q V E F M L P V A H T V R L I R V T S T S A S Q G A S : 128
VR2332 : FTPEER Q L C L S I O T A F N Q G A G T C T L D S G R L S Y T V E F S L P T H T H T V R L I R V T S A S P S A --- : 123
```

LV : TTAAACACTCA-----GGTGAATGGCCGCGATTGGCG : 32  
VR2332 : TGGCGTGGCATCTCTGAGGCATCTCAGTGTTTGAATGGAGAATGTGTGTTGAATGGCACTGATTGACA : 70  
          \* \* \* \* \*  
          \*\*\*\*\*

LV : TGTGGCCTCTGAGTCACCTATTCAATTAGGGCGATCACATGGGGTCATACTTAATCAGCAGGAACCAT : 102  
VR2332 : TTGTGGCCTCTAAGTCACCTATTCAATTAGGGCGACCGTGTGGGGTGAGATTTAATT-GCGGAGAAACCAT : 139

LV : GTGACCGAAATTAAAAAAA: 122  
VR2332 : GCGCCGAAATTAAAAAAA: 159  
\* \* \* \* \*

6.  
Li.

TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije

Docket No.: 2183-6217US

8/13

| Constructs | Deletion<br>(nucleotides /<br>amino acids)  | Plasmid<br>number   | M-<br>expression  | N-<br>expression  | Virus<br>production   |
|------------|---|---|---|---|---|
|            | <p>wild type</p> <p>Δ14975-14980 / Δ2</p> <p>Δ14969-14980 / Δ4</p> <p>Δ14966-14980 / Δ5</p> <p>Δ14963-14980 / Δ6</p> <p>Δ14960-14980 / Δ7</p> <p>Δ14957-14980 / Δ8</p> <p>Δ14954-14980 / Δ9</p> <p>Δ14989-14995</p> <p>Δ14989-15020</p> | <p>437</p> <p>639</p> <p>694</p> <p>745</p> <p>746</p> <p>747</p> <p>748</p> <p>695</p> <p>693</p> <p>729</p> | <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>-</p> | <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>-</p> | <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>-</p> <p>-</p> <p>-</p> <p>+</p> <p>-</p> |

Fig. 7



TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije

Docket No.: 2183-6217US

9/13

Growth curves of PRRSV deletion mutants

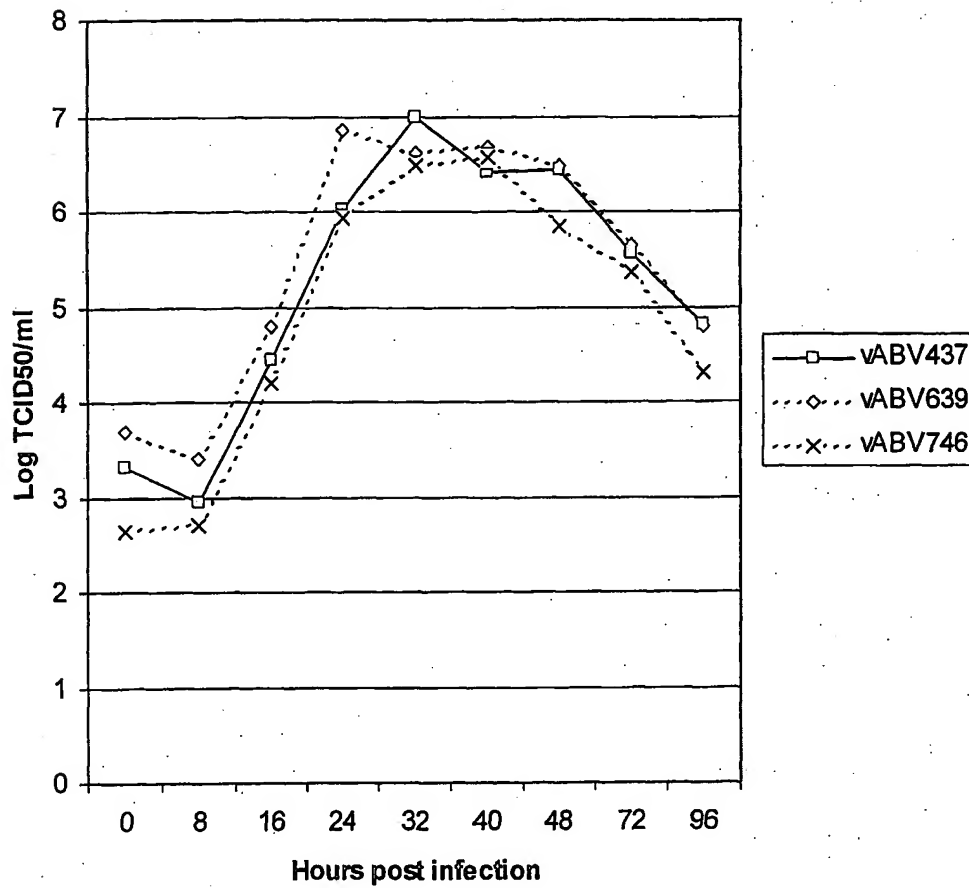


Fig. 8

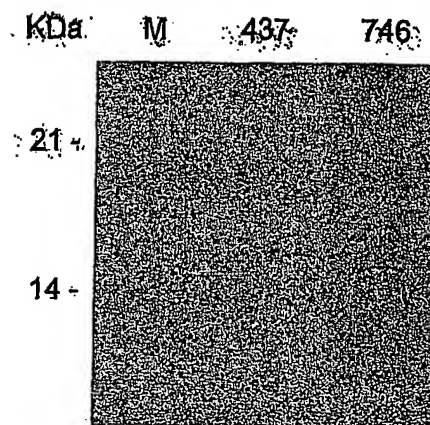
TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije

Docket No.: 2183-6217US

10/13

Fig. 9



BEST AVAILABLE COPY

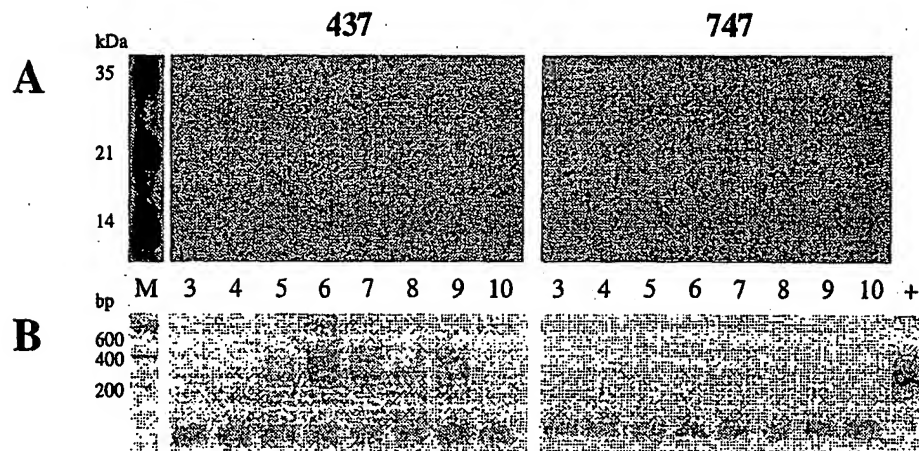
TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije

Docket No.: 2183-6217US

11/13

Fig. 10



BEST AVAILABLE COPY

12/13

TABLE 1: Sequences of the primers used to introduce deletions by PCR, and primers used to sequence the introduced mutations.

| Primer   | Sequence of the primer <sup>a</sup>                   | Orientation | Purpose (pABV) | Location |
|----------|---|-------------|----------------|----------|
| 119R218R | 5' ATGACATCCGGCACCACC 3'                              | +           | Sequencing     | 14782    |
| LV20     | 5' CCTGATTAAAGCTTGACCCC 3'                            | -           | Sequencing     | 15066    |
| LV75     | 5' TCTAGGAATTCTAGACGATCG 3'                           | -           | XbaI -site     | 15088    |
| LV155    | 5' ACGTGCCTTAACCTCGTCAAGTATGGCCGGTAAAAACCAGAGCCAGA 3' | +           | HpaI -site     | 14582    |
| LV204    | 5' ACGTGCCTTAATTAACCTTGAATGGCGGATGTAGA 3'             | -           | 639            | 14974    |
| LV213    | 5' TGCAAGTTAATTAAGGTGAATGGCCGCGA 3'                   | +           | 693            | 14996    |
| LV214    | 5' GACTGTTTAACTGGCGGATGTA 3'                          | -           | 694            | 14958    |
| LV215    | 5' GACTGTTTAAATTAATGTCACGCGAATC 3'                    | -           | 695            | 14942    |
| LV239    | 5' TGCAAGTTAATTAAGCCTCTGAGTCA 3'                      | +           | 729            | 15021    |
| LV263    | 5' GACTGTTTAAATTAAGCGGATGTAGA 3'                      | -           | 745            | 14954    |
| LV264    | 5' GACTGTTTAAATTAAGTGTAGAAATC 3'                      | -           | 746            | 14951    |
| LV265    | 5' GACTGTTTAAATTAAGTGAAGTCACG 3'                      | -           | 747            | 14948    |
| LV266    | 5' GACTGTTTAAATTAAGAGTCACGCGA 3'                      | -           | 748            | 14945    |

<sup>a</sup> The restriction sites are underlined.

Fig. 11

TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije  
Docket No.: 2183-6217US

13/13

Fig. 12

TABLE 1: Sequences of the primers used to introduce deletions by PCR, primers used to sequence the introduced mutations, and primers used for the strand-specific RT-PCR

| Primer  | Sequence of the primer <sup>a</sup>                         | Orien-<br>tation | Purpose<br>(pABV)           | Location |
|---------|---|------------------|-----------------------------|----------|
| 118U750 | 5' CAGCCAGGGGAAATGTGGC 3'                                   | -                | Sequencing / Strand-sp. PCR | 14745    |
| 12U94R  | 5' CACCTGTACCTGCTCAATTGT 3'                                 | -                | Strand-sp. PCR              | 2334     |
| 23U101  | 5' GTTCTAGCCCAACAGGTATC 3'                                  | +                | Strand-sp. RT               | 85       |
| LV2     | 5' AGCGGGAAGGATCCACAGGTAT 3'                                | +                | Strand-sp. PCR              | 147      |
| LV17    | 5' CCCTTGACGAGCTCTTCGGC 3'                                  | +                | Sequencing                  | 14045    |
| LV20    | 5' CCTGATTAAAGCTTGACCCC 3'                                  | +                | Sequencing                  | 15066    |
| LV75    | 5' TCTAGGAATCTAGACGATCG                                     | -                | PCR XbaI-site               | 15088    |
| LV76    | 5' TCTAGGAATCTAGACGATCG(T)40 3'                             | -                | RT                          | 15088    |
| LV78    | 5' CCTGGGATGAATCTATGT 3'                                    | -                | Strand-sp. RT               | 10306    |
| LV79    | 5' GACAAGATCATCAGAGTATACC 3'                                | -                | Strand-sp. PCR              | 8904     |
| LV84    | 5' AGAGCTTCAGGACACTGACC 3'                                  | +                | Strand-sp. RT               | 321      |
| LV112   | 5' CCAATTCACCTGACTGTCTTAACTTGCACCCCTGA 3'                   | -                | Strand-sp. PCR              | 14981    |
| LV118   | 5' TTACCACTACTCTCCACCG 3'                                   | +                | Strand-sp. PCR              | 1464     |
| LV132   | 5' CCTACTGTGCCCTATAGTTC 3'                                  | +                | Strand-sp. PCR              | 8023     |
| LV151   | 5' ACCAGGCGCAGAGAAAGAAAGTACAGCTGGGTGCAATGAT 3'              | +                | PCR (631)                   | 14611    |
| LV152   | 5' ACCAGGCGCAGAGAAAGAAAGTACAGCTGGGTGCTGG 3'                 | +                | PCR (632)                   | 14611    |
| LV153   | 5' ACCAGGCGCAGAGAAAGAAAGTACAGCTTCAATCACTGT 3'               | +                | PCR (633)                   | 14611    |
| LV154   | 5' ACCAGGCGCAGAGAAAGAAAGTACAGCTTATGGCCAGCCAG 3'             | +                | PCR (634)                   | 14611    |
| LV155   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGAACAGAGCCAGA 3'        | +                | HpaI-site PCR               | 14582    |
| LV188   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | +                | PCR (602)                   | 14582    |
| LV189   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | +                | PCR (603)                   | 14582    |
| LV190   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | +                | PCR (604)                   | 14582    |
| LV191   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | +                | PCR (624)                   | 14582    |
| LV195   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | +                | PCR (625)                   | 14582    |
| LV196   | 5' GGAGTGGTAAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | +                | PCR (626)                   | 14582    |
| LV197   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | +                | NheI-site PCR               | 14140    |
| LV198   | 5' GCTCGTGAAGCTTTAGCATCACATACAC 3'                          | +                | PCR (635)                   | 14981    |
| LV200   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | -                | PCR (636)                   | 14981    |
| LV201   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | -                | PCR (637)                   | 14981    |
| LV202   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | -                | PCR (638)                   | 14981    |
| LV203   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | -                | PCR (639)                   | 14981    |
| LV204   | 5' ACGTGGCTTAAGCTCTGTAAGTATGGCGGTAAAGTCCCA 3'               | -                | PCR (696)                   | 14611    |
| LV216   | 5' ACCAGGCGCAGAGAAAGAAAGTACAGCTCCGATGGGGAG<br>GGTCAATGAT 3' | +                | PCR (769)                   | 14611    |
| LV268   | 5' ACCAGGCGCAGAGAAAGAAAGTACAGCTCCGATGGGGAG 3'               | +                | PCR (769)                   | 14641    |
| LV269   | 5' CTCGGATGGGATGGCCAGCCAGGTGTAAGAACTGTGCCAGT 3'             | +                | PCR (768)                   | 14981    |
| LV270   | 5' TGCAAGTTAATTAACAGTCAAGTGAATGGCCGCTAACCGTGTGGCCTC 3' +    | +                |                             |          |

<sup>a</sup> The restriction sites are underlined.